Electron Transfer Chemistry between DNA and DNA-Binding Tripeptides†

Elke Mayer-Enthart, Peter Kaden, and Hans-Achim Wagenknecht*

Chemistry Department, Technical University of Munich, Lichtenbergstr. 4, D-85747 Garching, Germany

Received March 11, 2005; Revised Manuscript Received June 10, 2005

ABSTRACT: A DNA system consisting of pyrene-modified oligonucleotides and nitrobenzoate (Nb)-modified DNA-binding tripeptides has been applied to study electron-transfer processes through the DNA−peptide interface. 5-(Pyren-1-yl)-2′-deoxyuridine (Py-dU) has been used as the photoinducible charge generator. Upon excitation at 350 nm, a pyrene-like excited state (Py-dU)* is formed which undergoes an electron transfer yielding the charge-separated state which is the contact ion pair $Py^{\bullet+}-dU^{\bullet-}$. The subsequent electron shift from $dU^{\bullet-}$ into the base stack competes with charge recombination and can be probed chemically by trapping the electron at the 5-bromo-2′-deoxyuridine (Br-dU) group leading to strand cleavage which can be quantified by HPLC analysis. Several Nb-modified DNA-binding tripeptides influence these DNA-mediated electron-transfer processes as shown by fluorescence spectroscopy experiments. Fluorescence quenching can occur primarily through a reductive electron-transfer process in which the Nb group traps the electron thermodynamically from the contact ion pair $Py^{\bullet+}-dU^{\bullet-}$. Moreover, our results indicate that, once the negative charge has been trapped on the peptide, oxidative processes from $Py^{\bullet+}$ take place resulting in an enhanced and nonspecific strand degradation of the Py-dU-modified duplexes. The latter type of strand cleavage can be inhibited by the presence of tryptophane or tyrosine as part of the peptides. Most remarkably, DNA-binding tripeptides, which bear both the Nb and the tryptophan/tyrosine moiety, are able to trap both the negative and the positive charge from the contact ion pair $Py^{\bullet+}-dU^{\bullet-}$.

The genome is continuously exposed to various sources for damage with severe consequences such as permanent mutations or cell death (*1*−*9*). Especially, oxidative DNA damage plays an important role in aging processes, for several diseases, and is a major source for cancer. In many cases, the formation of DNA damages represents the chemical consequence of the occurrence of DNA base radicals as charged precursors within the DNA duplex (*10*). Once a positively or negatively charged radical has been generated, the charge exhibits a mobility which could form a radical cation or anion at a distant site resulting in the irreversible formation of DNA lesions. Such DNA-mediated charge-transfer processes can be categorized as either oxidative hole transfer or reductive electron-transfer processes (*11*−*13*). Both processes represent, in principle, electron-migration reactions but with different orbital control; the oxidative hole transfer is HOMO-controlled, whereas the excess electron transfer is LUMO-controlled. This makes clear that this categorization is not just formalism about the different directions of the electron.

For the oxidative type of DNA-mediated charge-transfer processes, a significant relevance in the formation of oxidative damage to the DNA has been shown (*11*). Since guanine represents the DNA base with the lowest oxidation potential (*14*, *15*), it plays a special role during oxidative DNA damaging (*9*, *16*). The resulting oxidative guanine damages cause permanent mutations (*2*, *7*) or promote the aging process if they occur within the telomers (*5*, *17*). Remarkably, guanine oxidation can be repaired reductively or can lead to physiologically relevant DNA−protein cross-links and adducts (*18*−*21*).

On the other hand, excess electron-transfer processes play a growing role in the development of electrochemical DNA chips, for example, for the detection of single base mutations (*22*). In contrast to the broad knowledge about oxidative hole transfer in DNA for which the mechanisms have been elucidated in the 1990s (*11*, *23*), the reductive type of DNA-mediated charge transfer has been subject to intense research only in the last 3−4 years (*12*). It was proposed (*13*) and proven experimentally that during excess electron transfer each base pair can participate since the radical anions of C and T play the role as intermediate electron carriers. By now, no DNA lesions have been obtained as the irreversible chemical result of the occurrence of an excess electron in the DNA. The increasing knowledge about excess electron transfer in DNA support the idea that this type of charge transfer has the potential to be considered for the development of nanotechnological applications, such as new DNA-based electronic devices (*24*).

DNA-mediated charge transfer exhibits an extremely high sensitivity to disruptions of the π-stacking between the intervening DNA bases which are caused by perturbations of the DNA structure or conformation (*11*). DNA−protein interactions are of special interest since they occur in a highly site-selective fashion. In fact, DNA-mediated charge-transfer processes can be modulated both negatively and positively by DNA-binding proteins (*25*−*28*). Most importantly, each of the observed influences of the proteins can be explained by special structural features of the corresponding DNA−

† This work was supported by the Volkswagen-Stiftung (Az I/78 101), the Deutsche Forschungsgemeinschaft, and the Fonds der Chemischen Industrie.

* To whom correspondence should be addressed: Tel: 49-89-289-13303. Fax: 49-89-289-13210. E-mail: Wagenknecht@ch.tum.de.

10.1021/bi0504557 CCC: $30.25
Published on Web 08/09/2005

protein complexes, but a profound and clear understanding of the electronic coupling between proteins and DNA is still missing. For this purpose, DNA-binding peptides could serve as suitable models to elucidate characteristic DNA−protein interactions electronically.

To study the DNA-mediated electron-transfer chemistry in a photoinduced fashion, it is crucial to modify oligonucleotides with suitable chromophores (*29*, *30*). Recently, we used pyrene (*31*−*36*), ethynylpyrene (*37*−*39*), phenothiazine (*40*), and ethidium (*41*−*43*) as photoinducible electron donors. The electron-transfer processes have been studied by steady-state fluorescence spectroscopy, time-resolved laser spectroscopy, and chemical probing. We found that DNA has to be considered as a structurally flexible medium and DNA-mediated electron transfer cannot be reduced to a static donor−bridge−acceptor situation (*36*). This interpretation stands in agreement with the most recent results about the mechanism of oxidative hole transfer (*44*, *45*). In particular, there is strong experimental evidence for an involvement of base-stacking fluctuations and hydrogen-bonding interactions (not necessarily proton transfer) inside the DNA helix. With respect to these observations, the influence of DNA ligands becomes increasingly important for the understanding of the mobility of radical charges in the DNA. In principle, interactions between DNA bases and amino acids as part of DNA-binding proteins or peptides influence the DNA dynamics and hence participate potentially in DNA-mediated charge-transfer processes.

Herein, we want to present our recent efforts to investigate electron-transfer processes through the DNA−peptide interface. The DNA system consists of duplex DNA equipped with pyrene as the electron donor and DNA-binding peptides bearing a nitrobenzoate group as the electron acceptor. The electron transfer has been explored by fluorescence quenching experiments as well as chemical probing.

EXPERIMENTAL PROCEDURES

Materials and Methods. Chemicals were purchased from Lancaster, Aldrich, and Fluka; protected amino acids (Fmoc-Lys(Boc)-OH, Fmoc-Tyr(*t*Bu)-OH, Fmoc-Trp-OH) were purchased from Novabiochem. They were used without further purification. Solvents were distilled before use and dried according to standard procedures. ESI mass spectra were measured in the analytical facility of the institute on a Finnigan LCQ-ESI spectrometer. MALDI−TOF analysis was performed on a Bruker Biflex III spectrometer using 3-hydroxypicolinic acid in aqueous ammonium citrate as the matrix. RP-C18 analytical and semipreparative HPLC columns (300 Å) were purchased from Supelco. All spectroscopic measurements were performed in quartz glass cuvettes (1 cm) and using Na−P_i-buffer (10 mM). Absorption spectra (5 μM duplex) were recorded on a Varian Cary Bio 100 spectrometer. Fluorescence spectra (5 μM duplex, $\lambda_{exc} = 360$ nm) were recorded on a Fluoromax-3 fluorimeter (Jobin-Yvon) and corrected for Raman emission from the buffer solution. All emission spectra were recorded with a bandpass of 2 nm for both excitation and emission and were intensity corrected. The corrected fluorescence intensity of the titration experiments was integrated from 390 to 630 nm and normalized. The CD spectroscopy (2.5 μM duplex, 185−400 nm) was performed on a Jasco J-715 spectropolarimeter.

Preparation of Oligonucleotides (General Procedure). The oligonucleotides were prepared on an Expedite 8909 DNA synthesizer (Applied Biosystems) via standard phosphoramidite protocols using CPGs (1 μmol) and chemicals from ABI and Glen Research. After preparation, the trityl-off oligonucleotide was cleaved off the resin and was deprotected by treatment with concentrated NH_4OH at 55 °C for 10 h. The oligonucleotide was dried and purified by HPLC on a semipreparative RP-C18 column using the following conditions: A = NH_4OAc buffer (50 mM), pH 6.5; B = MeCN; gradient = 0−15% B over 45 min. The oligonucleotides were lyophilised and quantified by their absorbance at 260 nm (*46*).

Preparation and Characterization of Modified Oligonucleotides. The oligonucleotides were prepared on an Expedite 8909 DNA synthesizer (Applied Biosystems) via standard phosphoramidite protocols. The Py-dU[1] building block (*31*) and the Br-dU building block (ChemGenes) coupled nearly quantitatively. After preparation, the trityl-off oligonucleotides were treated with concentrated NH_4OH at room temperature for 27 h, protected from light. The oligonucleotides were dried and purified by HPLC on a semipreparative RP-C18 column using the following conditions: A = NH_4-OAc buffer (50 mM), pH 6.5; B = MeCN; gradient = 0−30% B over 45 min. The oligonucleotides were lyophilised and quantified by their absorbance at 260 nm (*46*), using ϵ (260 nm) = 14.600 M^{-1} cm^{-1} for Py-dU (*31*) and ϵ (260 nm) = 7.000 M^{-1} cm^{-1} for Br-dU (ChemGenes). MS (MALDI−TOF): DNA1 (ss) *m*/*z* (calcd) 5339, *m*/*z* (exp) 5347; DNA2 (ss) *m*/*z* (calcd) 5306, *m*/*z* (exp) 5309; DNA3 (ss) *m*/*z* (calcd) 5393, *m*/*z* (exp) 5392.

Preparation and Characterization of Modified Peptides. After swelling with dry CH_2Cl_2 (10 mL) for 1 h, the TCP resin (1.00 g, 0.95 mmol/g) was treated with a solution of Fmoc-protected amino acids (1.2 equiv) in dry CH_2Cl_2 (10 mL) and Hünig's base (3 equiv) at room temperature for 1 h. MeOH (1 mL) was added as a capping agent, and the reaction mixture was shaken for 15 min. The resin was washed with CH_2Cl_2 (3 × 10 mL), NMP (5 × 10 mL), and MeOH (5 × 10 mL) and dried under vacuum. The resin was suspended three times in a solution of 20% piperidine in NMP (10 mL), agitated for 10 min, and washed with NMP (5 × 10 mL). The Fmoc-amino acid (2 equiv) in a 0.2 mM TBTU (2 equiv) and HOBt (2 equiv) solution in NMP and DIPEA (5.6 equiv) was added to the resin. The reaction mixture was shaken at room temperature for 1 h and washed with NMP (5 × 10 mL). In case of *p*-nitrobenzoate-modified peptides, *p*-nitrobenzoic acid has been coupled terminally using a reaction time of 2 h. Finally, the resin was washed with CH_2Cl_2 (5 × 10 mL) and treated with a solution of TFA/H_2O/triisopropylsilane 95:1:4 (10 mL) for 3 × 1 h. After removal of the resin by filtration, the filtrates were combined and the solvent was removed in vacuo. The peptide was precipitated in cold Et_2O to yield the trifluoracetic salt of the peptide as a light yellow powder. ESI-MS: *m*/*z* (%); Lys-Lys-Lys 403.6 (100) $[M + H]^+$, 202.5 (10) $[M + 2H]^{+2}$; Lys-Tyr-Lys 438.4 (100) $[M + H]^+$, 219.9 (30) $[M + 2H]^{+2}$; Lys-Trp-Lys 921.3 (40) $[2M + H]^+$, 461.4 (100) $[M + H]^+$, 231.4 (30) $[M + 2H]^{+2}$; Nb-Lys 296.2 (100) $[M + H]^+$;

[1] Abbreviations: CIP, contact ion pair; Nb, nitrobenzoate; Py, pyrene; Py-dU, 5-(pyren-1-yl)-2′-deoxyuridine.

Nb-Lys-Lys 552.4 (100) $[M + H]^+$, 276.9 (30) $[M + 2H]^{+2}$; Nb-Lys-Lys-Lys 587.5 (100) $[M + H]^+$, 294.4 (40) $[M + 2H]^{+2}$; Nb-Lys-Tyr-Lys 1219.4 (20) $[2M + H]^+$, 610.4 (100) $[M + H]^+$, 305.9 (70) $[M + 2H]^{+2}$; Nb-Lys-Trp-Lys 847.2 (20) $[2M + H]^+$, 424.3 (100) $[M + H]^+$, 233.0 (60) $[M + 2H]^{+2}$.

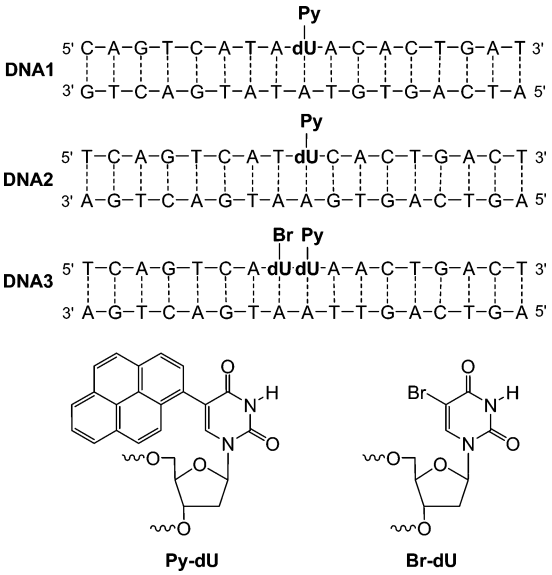
For spectroscopic measurements, the peptides were dissolved in H_2O and quantified by their absorbance: ϵ (270 nm) = 16.000 M^{-1} cm^{-1}(Nb), ϵ (270 nm) = 5.400 M^{-1} cm^{-1}(Trp), ϵ (260 nm) = 1.400 M^{-1} cm^{-1} (Tyr) (*47*).

Strand Cleavage Experiments. Duplexes (4 μM DNA, 10 mM Na−P_i-buffer, 250 mM NaCl) were prepared by heating equimolar solutions of the single strands to 90 °C for 10 min in the dark and subsequent slow cooling. The measurements were performed in quartz glass cuvettes (1 cm). The freshly prepared duplexes were irradiated by a Xenon lamp (75 W Xe lamp, 14 V, 5.4 A; Oriel Instruments) equipped with a cutoff filter (Andover Corporation, >305 nm). Every 5 min, aliquots (30 μL) of the sample solution (1000 μL) were taken to a RNAse/DNAse-free container and stored protected from light at room temperature. After the withdrawal of the last sample after 60 min, piperidine (3 μL) was added to all samples. The samples were heated to 90 °C for 30 min, lyophilised, dissolved in water (15 μL), and analyzed by analytical HPLC (gradient = 0−30% B). The obtained peaks were processed to give a ratio between unmodified and modified DNA-single strand.

RESULTS

Design, Preparation, and Energetics in the Py-dU−DNA System. By now, our group focused mainly on pyrene (Py) as the photoexcitable electron donor for the investigation of reductive electron transfer in nucleosides and DNA (*31*−*36*). Pyrene-labeled oligonucleotides were used previously by Netzel and co-workers to investigate the quenching efficiency of the photoexcited pyrene emission (Py*) depending on the flanking DNA bases (*48*). Emission spectra and lifetime measurements provided evidence for an electron transfer occurring from Py* to the pyrimidine bases T/C and a hole transfer from Py* to the purine base G. This charge-transfer assignment has been established by nanosecond fluorescence lifetime measurements with 5-(pyren-1-yl)-2′-deoxyuridine (Py-dU) (*49*, *50*) and by picosecond transient absorption experiments using benzopyrenyl-2′-deoxyguanosine conjugates (*51*, *52*). With respect to these redox assignments, we chose to attach a pyrene group covalently to cytidine and thymidine as the bases which were proposed to be relevant as intermediate charge carriers during reductive electron transfer in DNA (*13*). In the case of Py-dU and 5-(pyren-1-yl)-2′-deoxycytidine (Py-dC), excitation of the pyrene moiety leads to an intramolecular electron transfer yielding the corresponding pyrimidine radical anions ($Py^{\bullet+}$−$dU^{\bullet-}$ and $Py^{\bullet+}$−$dC^{\bullet-}$) (*32*, *35*). In accordance with our studies, both dU (which is structurally similar to T) and C are significantly weaker electron acceptors than expected based upon redox potentials. Combining the potentials $E(Py^{\bullet+}/Py^*)$ = −1.8 V (vs NHE) (*53*) and $E(dU/dU^{\bullet-})$ = $E(C/C^{\bullet-})$ = −1.1 V (*54*), the driving force ΔG for the ET process in Py-dU or Py-dC could be maximal −0.6 eV (using E_{00} = 3.25 eV for Py* (*53*)). However, our studies revealed a driving force $\Delta G \approx 0$ eV which requires the potential

Scheme 1: Sequences of the Py-dU-Modified DNA Duplexes **1**−**3**



$E(dU/dU^{\bullet-})/E(C/C^{\bullet-})$ to be ∼−1.8 V (*32*, *35*). Both $C^{\bullet-}$ and $T^{\bullet-}$ have been postulated as intermediates during electron hopping in DNA (*13*). We found that $T^{\bullet-}$ is more likely to play a role as an electron carrier in DNA due to a proton-coupled electron transfer in case of $C^{\bullet-}$ (*33*, *35*, *55*). Hence, we chose the Py-dU-modified DNA to probe the electron transfer through the DNA base stack (*31*, *36*) and through the DNA−peptide interface.

Using our previously published synthetic procedures, we prepared the Py-dU-modified DNA duplexes **1**−**3** (Scheme 1) (*31*, *34*). DNA **1** and **2** contain Py-dU as the single modification and electron injection site. In these two DNA duplexes, C and T represent the closest electron acceptors for the subsequent electron shift from $dU^{\bullet-}$ and are placed either directly adjacent to the Py-dU group (DNA **1**) or separated by one intervening A−T base pair (DNA **2**). DNA **3** contains the 5-bromo-2′-deoxyuridine (Br-dU) label as the second modification representing a chemical trapping site for the excess electron (*36*, *40*, *56*−*60*).

The 4-nitrobenzoate (Nb) moiety represents a suitable thermodynamic electron trap for peptides because of its reduction potential ($E(Nb/Nb^{\bullet-})$ = −0.46 V) (*61*, *62*). Moreover, the Nb group can be easily attached to the N-terminus of peptides after the solid-phase synthesis via the Fmoc strategy. The smallest possible DNA-binding peptides Lys-Trp-Lys and Lys-Tyr-Lys have been investigated by Hélène et al. during the 1980s (*63*−*66*). These peptides bind primarily through the electrostatic interactions of the positively charged amino groups of the lysine side chains with the negatively charged phosphodiester backbone of the DNA. Additionally, these peptides bear an aromatic moiety capable of intercalation into the DNA double helix. The binding constants can be found in the range of $2-6 \times 10^{-4}$ M. According to these earlier results, we used the Nb-modified tripeptides Nb-Lys-Lys-Lys, Nb-Lys-Trp-Lys, and Nb-Lys-Tyr-Lys as the electron acceptor peptides in our Py-dU−DNA system. To show that the Nb-modification does not interfere with binding to the Py-modified DNA, we representatively performed differential CD spectroscopy of samples containing DNA **1** together with 20 equiv Nb-Lys-

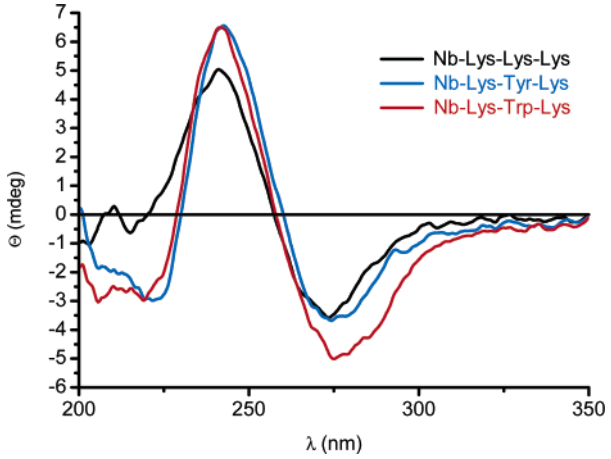


FIGURE 1: Differential CD spectroscopy of the Py-dU-modified DNA **1** (2.5 μM) in the presence of Nb-Lys-Lys-Lys, Nb-Lys-Trp-Lys, and Nb-Lys-Tyr-Lys (each 50 μM) in buffer (10 mM Na−P_i and 250 mM NaCl, pH 7).

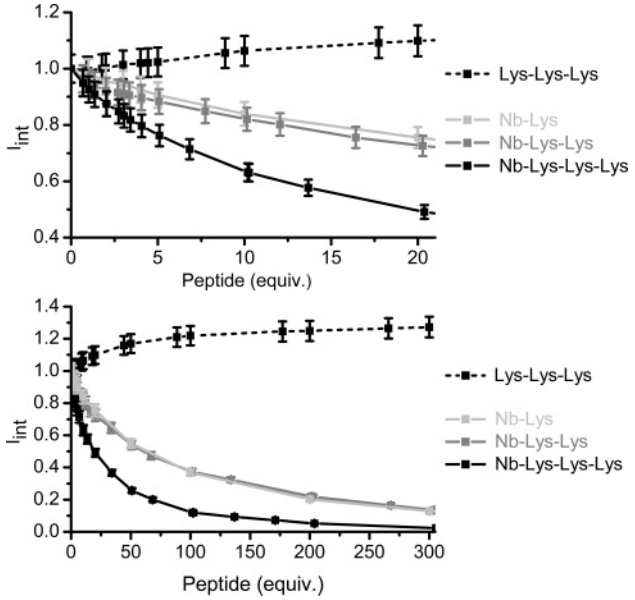


FIGURE 2: Fluorescence quenching experiments of the Py-dU-modified DNA **1** (5.0 μM) in the presence of Lys-Lys-Lys, Nb-Lys, Nb-Lys-Lys, and Nb-Lys-Lys-Lys in buffer (10 mM Na−P_i and 250 mM NaCl, pH 7).

Lys-Lys, Nb-Lys-Trp-Lys, or Nb-Lys-Tyr-Lys, respectively. After subtraction of the CD spectra of DNA **1** (without any peptide) as well as that of the peptides (without DNA), the corresponding CD spectra (Figure 1) show a significant Cotton effect around 260 nm which indicates that the Nb-modified tripeptides bind to DNA.

Fluorescence Quenching Experiments. As shown recently, the efficiency of the electron transfer after the electron injection in Py-dU-modified DNA duplexes drops significantly by the presence of just one intervening base pair (*36*). Hence, DNA **1** represents the best duplex for fluorescence quenching experiments since it exhibits a high emission (relative to DNA **2**) due to the fact that T or C are not placed directly adjacent to Py-dU as the electron donor group. First, we focused on the influence of the electrostatic interactions between the positively charged amino side chains of lysine and the negatively charged phosphodiester backbone of the DNA duplex. In fluorescence titration experiments, we applied the peptides Nb-Lys, Nb-Lys-Lys, and Nb-Lys-Lys-Lys bearing either one, two, or three positive charges, respectively, for potential DNA interactions (Figure 2). The unmodified peptide Lys-Lys-Lys was used as a reference lacking the electron acceptor Nb. Interestingly, in the latter case, the amount of fluorescence increases slightly with increasing peptide concentration indicating a stiffening of the DNA conformation which obviously results in an increased fluorescence of the pyrene moiety. In the case of the three Nb-modified-peptides, the fluorescence of the Py-dU group drops significantly with increasing peptide concentration. With respect to the control experiment using Lys-Lys-Lys, the fluorescence quenching can be attributed to the presence of the Nb group as the electron acceptor. Interestingly, the amount of fluorescence quenching of Nb-Lys/Nb-Lys-Lys is very similar, whereas Nb-Lys-Lys-Lys shows an increased quenching efficiency. It becomes clear that the third amino acid enhances the DNA-binding properties significantly, which stands in agreement with the known binding properties of small DNA-interacting peptides as elucidated earlier by Hélène et al. (*63*−*66*).

In a second set of Nb-modified peptides, the influence of intercalation as a second type of interaction between DNA and peptides was explored. Accordingly, the aromatic amino side chains of Trp and Tyr were incorporated into Nb-modified tripeptides which could potentially intercalate their indole or phenole side chains into the DNA base stack. The unmodified tripeptides Lys-Trp-Lys and Lys-Tyr-Lys have been applied as control peptides lacking Nb as the electron acceptor group. In fluorescence titration experiments, Nb-Lys-Tyr-Lys does not show any significant difference in its fluorescence quenching behavior (Figure 3) compared to that of Nb-Lys-Lys (which also bears two positively charged side chains). Hence, the intercalation of the phenole moiety does not enhance the electron-transfer capabilities between the Py-dU−DNA and the peptide. In contrast, Nb-Lys-Trp-Lys exhibits a slight increase of the amount of fluorescence quenching compared to Nb-Lys-Tyr-Lys and Nb-Lys-Lys. Interestingly, such a difference is also present in comparison between the fluorescence quenching properties of the unmodified tripeptides Lys-Tyr-Lys and Lys-Trp-Lys, which indicates a special electronic role of the indole side chain.

Chemical Probing. Br-dU has been established as a chemical electron trap for DNA-mediated electron-transfer reactions since it undergoes a chemical modification after its one-electron reduction which can be analyzed by piperidine-induced strand cleavage (*56*, *57*). Hence, the quantification of the strand cleavage yields information about the ET efficiency (*36*, *40*, *58*−*60*). Theoretical studies showed that the electron affinity of Br-dU is significantly higher compared to T (*67*, *68*). However, on the basis of reduction potentials, Br-dU seems not to be a significantly better electron acceptor (*69*). In conclusion, Br-dU represents more characteristics of a kinetic than a thermodynamic electron trap.

DNA **3** bears Br-dU as the chemical electron trap directly adjacent to the Py-dU group as the site of electron injection. As mentioned already above, the efficiency of the electron transfer which follows the electron injection in Py-dU-modified DNA duplexes drops significantly by the presence of just one intervening base pair (*36*). Hence, in contrast to the previously described fluorescence quenching measure-

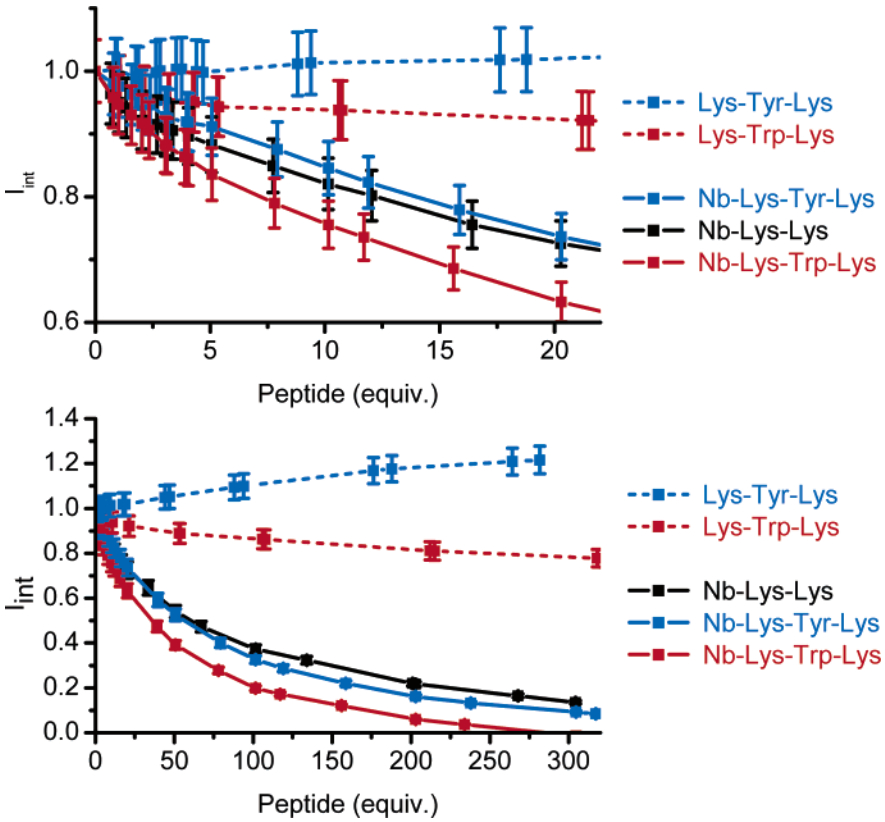


FIGURE 3: Fluorescence quenching experiments of the Py-dU-modified DNA **1** (5.0 μM) in the presence of Lys-Tyr-Lys, Lys-Trp-Lys, Nb-Lys-Lys, Nb-Lys-Tyr-Lys, and Nb-Lys-Trp-Lys in buffer (10 mM Na−P_i and 250 mM NaCl, pH 7).

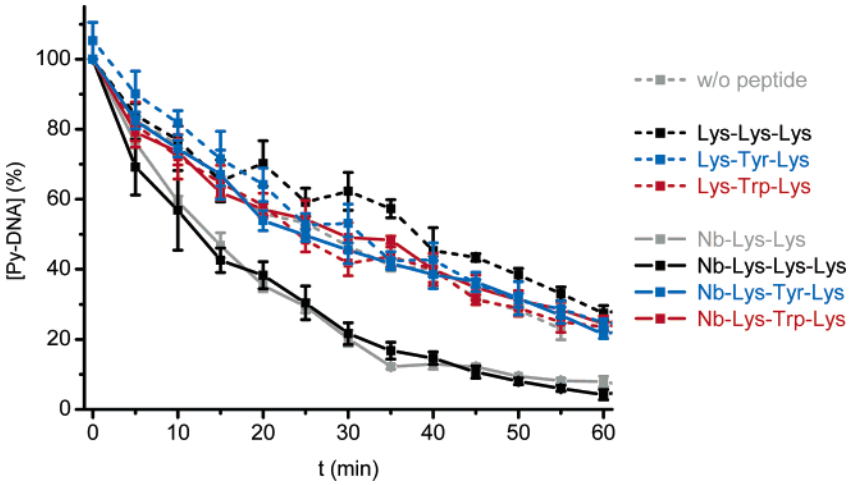


FIGURE 4: Analysis of the strand cleavage experiments with DNA **3** (4 μM) in the presence of Lys-Tyr-Lys, Lys-Trp-Lys, Nb-Lys-Lys, Nb-Lys-Tyr-Lys, and Nb-Lys-Trp-Lys in buffer (10 mM Na−P_i and 250 mM NaCl, pH 7). Each experiment has been repeated at least three times.

ments, DNA **3** represents the best duplex for chemical probing of the electron-transfer reaction since it exhibits a relatively high cleavage efficiency. The DNA **2** serves as a reference duplex lacking Br-dU as the electron acceptor for control experiments.

The irradiation experiments of DNA duplexes **2** and **3** have been performed in such a way that after the start of the experiment aliquots have been collected every 5 min, subsequently treated with piperidine at elevated temperature, and finally analyzed by HPLC. Each experiment had to be repeated at least three times. A 75 W Xe lamp with a cutoff filter (>305 nm) has been used for these experiments to selectively photoexcite the Py-dU chromophore and to avoid any degradation of the oligonucleotides by irradiation at smaller wavelengths. No strand cleavages have been observed during the irradiation of DNA **2** without the addition of any peptide (Figure 5). Initially, this experiment represented an important control that the observed strand cleavage in the DNA **3** can be assigned to the presence of Br-dU as the electron acceptor (Figure 4).

The unmodified tripeptides Lys-Lys-Lys, Lys-Tyr-Lys, and Lys-Trp-Lys did not show any influence of the strand degradation behavior during the irradiation of DNA **3**. As shown by the fluorescence quenching experiments, see above, only Nb-modified DNA-binding peptides are capable to trap the excess electron which was initiated by the photoinduced

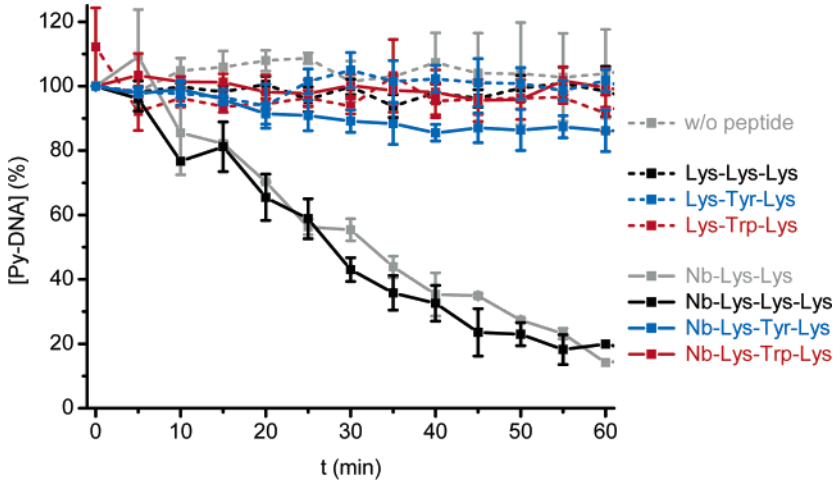


FIGURE 5: Analysis of the strand cleavage experiments with DNA **2** (4 μM) in the presence of Lys-Tyr-Lys, Lys-Trp-Lys, Nb-Lys-Lys, Nb-Lys-Tyr-Lys, and Nb-Lys-Trp-Lys in buffer (10 mM Na$-$P$_i$ and 250 mM NaCl, pH 7). Each experiment has been repeated at least three times.

charge separation in the Py-dU chromophore. Thus, it was expected that in the presence of Nb-modified peptides the strand degradation during the irradiation of DNA **3** should be decreased in comparison to experiments without the addition of such peptides. Surprisingly, the Nb-modified peptides Nb-Lys-Lys and Nb-Lys-Lys-Lys did enhance the efficiency of the strand cleavage (Figure 4). Even more unexpected was the observation that the Nb-modified tripeptides which bear an aromatic amino acid, Nb-Lys-Tyr-Lys and Nb-Lys-Trp-Lys, did not show an increased strand degradation of DNA **3** in comparison to the experiment without the addition of any peptide. It is important to point out that these peptides exhibit significant fluorescence quenching when added to DNA **1** showing that an electron transfer occurs indeed. These results indicate that there must be a second reaction channel which is different to the electron transfer from the Py-dU to the Br-dU group, but yields also strand degradation during the irradiation. It looked reasonable to assume that such a second reaction channel is inhibited in the presence of the indole or phenole group as part of the aromatic amino acids Trp or Tyr, respectively.

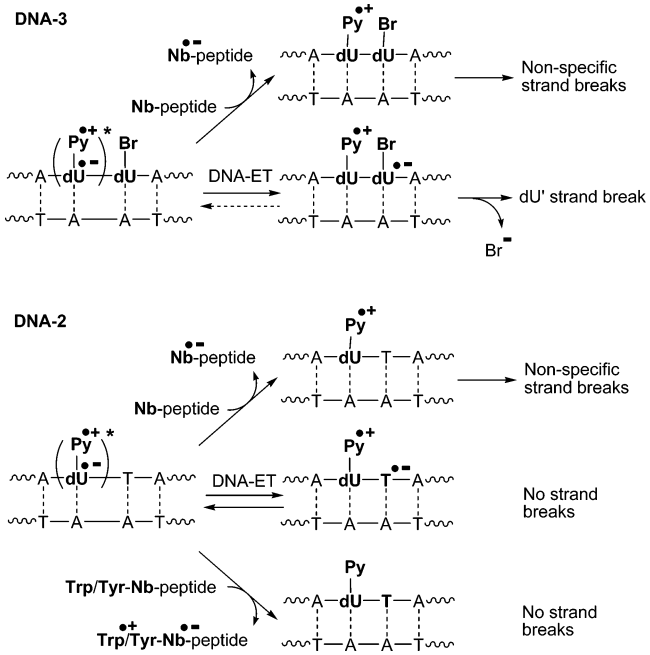
To explore these unexpected reactivities, further irradiation experiments were performed using DNA **2** which lacks the Br-dU group as the electron acceptor (Figure 5). As expected for this reference duplex, no strand degradation was observed without the addition of any peptide and also in the presence of the unmodified peptides Lys-Lys-Lys, Lys-Trp-Lys, and Lys-Tyr-Lys. Surprisingly, the irradiation of DNA **2** in the presence of Nb-Lys-Lys and Nb-Lys-Lys-Lys resulted in very efficient strand degradation. HPLC analysis of these irradiated samples indicated a variety of products and not a clear strand cleavage at one site as it was observed in case of the Br-dU-modified DNA **3**. As already indicated by the experiments with DNA **3**, the results with DNA **2** showed that there has to be a reaction channel other than reductive electron transfer which yields strand degradation during the irradiation. The presence of aromatic side chains in Nb-Lys-Trp-Lys and Nb-Lys-Tyr-Lys seems to inhibit this strand degradation.

DISCUSSION

In the Py-dU-modified DNA, a pyrene-like excited state (Py-dU)* is formed upon excitation at 350 nm, which undergoes an electron transfer yielding the charge separated state Py$^{\bullet+}-$dU$^{\bullet-}$. In accordance with our recently published transient absorption experiments (*36*, *70*), the charge-separated state shows, in addition to the local pyrenyl absorption bands (Py* and Py$^{\bullet+}$), a broad and intense band in the near-infrared region which corresponds to a contact ion pair (CIP) absorption which is a composite of the two strongly interacting π-systems in Py$^{\bullet+}-$dU$^{\bullet-}$. This exciplex state shows fluorescence. The corresponding emission was measured in the experiments presented herein. In contrast to local radical ion bands, the intensity of the CIP band depends on the magnitude of the electronic coupling between the two aromatic subsystems and therefore on the conformation of Py-dU. The subsequent electron shift from dU$^{\bullet-}$ into the base stack competes with charge recombination as we have shown previously by combining spectroscopic and chemical methods (*31*, *32*, *35*, 36). Only this subsequent electron-shift process quenches the pyrene emission and can be probed chemically by trapping the electron at the Br-dU group leading to strand cleavage which can be quantified by HPLC analysis (Scheme 2). The applied Nb-modified DNA-binding peptides can influence this DNA-mediated electron-transfer processes as shown by the fluorescence titration experiments (Figures 2 and 3). Fluorescence quenching can occur primarily through a reductive electron-transfer process in which the Nb group traps the electron from the CIP state. Strong electrostatic interactions facilitate this electron trapping as it is observed in the order Nb-Lys < Nb-Lys-Lys < Nb-Lys-Lys-Lys.

Such a reductive electron-transfer process to the Nb group yields Py$^{\bullet+}$ with an oxidation potential E(Py$^{\bullet+}$/Py) of 1.4 V (*53*) capable to oxidize DNA (*15*). Hence, it is very likely that the increased nonspecific strand degradation which has been observed with DNA **3** and, more importantly, DNA **2** in the presence of Nb-Lys-Lys or Nb-Lys-Lys-Lys can be attributed to oxidative reactions which have been initiated by Py$^{\bullet+}$. Interestingly, the nonspecific irradiation damage of DNA **2** and **3** is not observed in the presence of the modified DNA-binding peptides Nb-Lys-Tyr-Lys and Nb-Lys-Trp-Lys bearing the aromatic amino acids tyrosine or tryptophane, respectively. Although the Nb group is present in these peptides which, according to the fluorescence quenching

Scheme 2: Summary of the Charge Transfer Processes Occurring between the Py-dU-Modified DNA and the Nb-Modified Peptides



properties, can trap the electron from the CIP state of Py-dU, the strand degradation is *not* enhanced in comparison to the corresponding irradiation experiments without the addition of peptides. Trp and Tyr contain electron-rich aromatic moieties ($E^0(Tyr^{\bullet+}/Tyr) = 0.9$ V (*71*), $E^0(Trp^{\bullet+}/Trp) = 1.0$ V (*15*)) and thus the corresponding peptides should be able to trap positive charges from the CIP state of Py-dU effectively. As a result, the nonspecific strand degradation of DNA **2** and DNA **3** is inhibited. As a consequence, our results indicate that DNA-binding tripeptides which bear both the Nb and the Trp/Tyr moiety are able to trap both the negative and the positive charge from the CIP state which is formed upon photoexcitation of the Py-dU chromophore in DNA.

CONCLUSIONS

We applied small Nb-modified DNA-binding peptides to elucidate the DNA−peptide interactions electronically. Py-dU-modified DNA has been used as the photoinducible charge generator. Upon excitation at 350 nm, the subsequent electron shift from the charge separated state $Py^{\bullet+}-dU^{\bullet-}$ into the base stack can be probed by chemical means using the Br-dU group as a kinetic electron trap. The Nb group serves as a thermodynamic trap for the electron transfer through the DNA−peptide interface. Our results indicate that, once the negative charge has been trapped in the peptide, oxidative processes from $Py^{\bullet+}$ take place resulting in an enhanced nonspecific strand degradation of the Py-dU-modified duplexes. This latter type of strand cleavage can be inhibited by the presence of Trp or Tyr as part of the peptides. It is likely that the positive charge is trapped from the charge-separated state $Py^{\bullet+}-dU^{\bullet-}$ by the indole or phenole side chain, respectively. Most remarkably, DNA-binding tripeptides which bear both the Nb and the Trp/Tyr moiety seem to be able to trap both the negative and the positive charge.

Although it is obvious that in biological systems neither Py-dU nor Nb are present, it is clear that our results indicate that DNA-binding peptides could influence the occurrence of charged DNA base radicals in the double helix significantly. DNA-binding peptides serve as models for DNA-binding proteins and are able to trap charges effectively from the DNA. Charge injection under physiological conditions could take place by the participation of protein cofactors, for example, flavines or Fe−S clusters, which are present in a variety of DNA-binding proteins such as transcription factors or repair enzymes (*72*). It is important to point out that, according to our results, charge-transfer processes can occur through the DNA−peptide interface. Interestingly, simple nonspecific interactions such as electrostatic interactions or intercalation between single amino acids in proteins and the DNA double helix provide the structural basis to trap positive and negative charges effectively out of the DNA. Hence, every DNA-binding protein which is present in biological systems, for example, histone in the cell nucleus, has a basic potential for protecting the genetic information from irreversible damage which could result from charged radicals as precursors.

SUPPORTING INFORMATION AVAILABLE

Figures showing HPLC analysis of DNA **2** or **3** after irradiation in the presence of Nb-Lys-Trp-Lys or Nb-Lys-Lys-Lys. This material is available free of charge via the Internet at http://pubs.acs.org.

REFERENCES

1. Becker, D., and Sevilla, M. D. (1993) The chemical consequences of radiation damage to DNA, *Adv. Radiat. Biol. 17*, 121−180.
2. Friedberg, E. C., McDaniel, L. D., and Schultz, R. A. (2004) The role of endogenous and exogenous DNA damage and mutagenesis, *Curr. Opin. Genet. Dev. 14*, 5−10.
3. Friedberg, E. C. (2003) DNA damage and repair, *Nature 421*, 436−440.
4. Hoeijmakers, J. H. J. (2001) Genome maintenance mechanisms for preventing cancer, *Nature 411*, 366−374.
5. Kawanashi, S., Hiraku, Y., and Oikawa, S. (2001) Mechanism of guanine-specific DNA damage by oxidative stress and its role in carcinogenesis and aging, *Mutat. Res. 488*, 65−76.
6. Poirier, M. C. (2004) Chemical-induced DNA damage and human cancer risk, *Nat. Rev. Cancer 4*, 630−637.
7. Wang, D., Kreutzer, D. A., and Essigmann, J. M. (1998) Mutagenicity and repair of oxidative DNA damage: insights from studies using defined lesions, *Mutat. Res. 400*, 99−115.
8. O'Neill, P., and Fielden, M. (1993) Primary free radical processes in DNA, *Adv. Radiat. Biol. 17*, 53−120.
9. Cadet, J., Berger, M., Douki, T., and Ravanat, J.-L. (1997) Oxidative damage to DNA: formation, measurement, and biological significance, *Rev. Physiol. Biochem. Pharmacol. 131*, 1−87.
10. Kawai, K., Takada, T., Nagai, T., Cai, X., Sugimoto, A., Fujitsuka, M., and Majima, T. (2004) Long-lived charge-separated state leading to DNA damage through hole transfer, *J. Am. Chem. Soc. 125*, 16198−16199.
11. Schuster, G. B. (2004) *Long-range charge transfer in DNA*, Topics in Current Chemistry, Vols. 236 and 237, Springer, Berlin, Germany.
12. Wagenknecht, H.-A. (2003) Reductive electron transfer and transport of excess electrons in DNA, *Angew. Chem., Int. Ed. 42*, 2454−2460.
13. Giese, B. (2002) Long-distance electron transfer through DNA, *Annu. Rev. Biochem. 71*, 51−70.
14. Seidel, C. A. M., Schulz, A., and Sauer, M. H. M. (1996) Nucleobase-specific quenching of fluorescent dyes. 1. Nucleobase one-electron redox potentials and their correlation with static and dynamic quenching efficiencies, *J. Phys. Chem. 100*, 5541−5553.
15. Steenken, S., and Jovanovic, S. V. (1997) How easily oxidizable is DNA? One-electron reduction potentials of adenosine and guanosine radicals in aqueous solution, *J. Am. Chem. Soc. 119*, 617−618.

16. Burrows, C. J., and Muller, J. G. (1998) Oxidative nucleobase modifications leading to atrand scission, *Chem. Rev. 98*, 1109−1151.
17. Delaney, S., and Barton, J. K. (2003) Charge transport in DNA duplex/quadruplex conjugates, *Biochemistry 42*, 14159−14165.
18. Copeland, K. D., Lueras, A. M. K., Stemp, E. D. A., and Barton, J. K. (2002) DNA cross-linking with metallointercalator−peptide conjugates, *Biochemistry 41*, 12785−12797.
19. Nguyen, K. L., Steryo, M., Kurbanyan, K., Nowitzki, K. M., Butterfield, S. M., Ward, S. R., and Stemp, E. D. A. (2000) DNA−protein cross-linking from oxidation of guanine via the flash-quench technique, *J. Am. Chem. Soc. 122*, 3585−3594.
20. Kurbanyam, K., Nguyen, K. L., To, P., Rivas, E. V., Lueras, A. M. K., Kosinski, C., Steryo, M., Gonzalez, A., Mah, D. A., and Stemp, E. D. A. (2003) DNA−protein cross-linking via guanine oxidation: dependence upon protein and photosensitizer, *Biochemistry 42*, 10269−10281.
21. Ly, A., Tran, N. Q., Sullivan, K., Bandonq, S. L., and Milligan, J. R. (2005) Involvement of proton transfer in the reductive repair of DNA guanyl radicals by aniline derivatives, *Org. Biomol. Chem. 3*, 917−923.
22. Drummond, T. G., Hill, M. G., and Barton, J. K. (2003) Electrochemical DNA sensors, *Nature Biotechnol. 21*, 1192−1199.
23. Jortner, J., Bixon, M., Langenbacher, T., and Michel-Beyerle, M. (1998) Charge transfer and transport in DNA, *Proc. Natl. Acad. Sci. U.S.A. 95*, 12759−12765.
24. Porath, D., Cuniberti, G., and Felice, R. D. (2004) Charge transport in DNA-based devices, *Top. Curr. Chem. 237*, 183−227.
25. Wagenknecht, H.-A., Rajski, S. R., Pascaly, M., Stemp, E. D. A., and Barton, J. K. (2001) Direct observation of radical intermediates in protein-dependent DNA charge transport, *J. Am. Chem. Soc. 123*, 4400−4407.
26. Rajski, S. R., Kumar, S., Roberts, R. J., and Barton, J. K. (1999) Protein-modulated DNA electron transfer, *J. Am. Chem. Soc. 121*, 5615−5616.
27. Rajski, S. R., and Barton, J. K. (2001) How different DNA-binding proteins affect long-range oxidative damage to DNA, *Biochemistry 40*, 5556−5564.
28. Nakatani, K., Dohno, C., Ogawa, A., and Saito, I. (2002) Suppression of DNA-mediated charge transport by BamHI binding, *Chem. Biol. 9*, 361−366.
29. Wagenknecht, H.-A. (2004) Synthetic oligonucleotide modifications for the investigation of charge transfer and migration processes in DNA, *Curr. Org. Chem. 8*, 251−266.
30. Grinstaff, M. W. (1999) How do charges travel through DNA?—an update on a current debate, *Angew. Chem., Int. Ed. 38*, 3629−3635.
31. Amann, N., Pandurski, E., Fiebig, T., and Wagenknecht, H.-A. (2002) Electron injection into DNA: synthesis and spectroscopic properties of pyrenyl-modified oligonucleotides, *Chem.—Eur. J. 8*, 4877−4883.
32. Amann, N., Pandurski, E., Fiebig, T., and Wagenknecht, H.-A. (2002) A model nucleoside for electron injection into DNA: 5-pyrenyl-2′-deoxyribose, *Angew. Chem., Int. Ed. 41*, 2978−2980.
33. Huber, R., Fiebig, T., and Wagenknecht, H.-A. (2003) Pyrene as a fluorescent probe for DNA base radicals, *Chem. Commun.* 1878−1879.
34. Mayer, E., Valis, L., Huber, R., Amann, N., and Wagenknecht, H.-A. (2003) Preparation of pyrene-modified purine and pyrimidine nucleosides via Suzuki−Miyaura cross-couplings and characterization of their fluorescent properties, *Synthesis*, 2335−2340.
35. Raytchev, M., Mayer, E., Amann, N., Wagenknecht, H.-A., and Fiebig, T. (2004) Ultrafast proton-coupled electron-transfer dynamics in pyrene-modified pyrimidine nucleosides: model studies towards an understanding of reductive electron transport in DNA, *ChemPhysChem 5*, 706−712.
36. Kaden, P., Mayer-Enthart, E., Trifonov, A., Fiebig, T., and Wagenknecht, H.-A. (2005) Real-time spectroscopic and chemical probing of reductive electron transfer in DNA, *Angew. Chem., Int. Ed. 44*, 1637−1639.
37. Rist, M., Amann, N., and Wagenknecht, H.-A. (2003) Preparation of 1-ethynylpyrene-modified DNA via Sonogashira-type solid-phase couplings and characterization of the fluorescence properties for electron-transfer studies, *Eur. J. Org. Chem.*, 2498−2504.
38. Mayer, E., Valis, L., Wagner, C., Rist, M., Amann, N., and Wagenknecht, H.-A. (2004) 1-Ethynylpyrene as a tunable and versatile molecular beacon for DNA, *ChemBioChem 5*, 865−868.
39. Wagner, C., Rist, M., Mayer-Enthart, E., and Wagenknecht, H.-A. (2005) 1-Ethynylpyrene-modified guanine and cytosine as optical labels for DNA hybridization, *Org. Biomol. Chem. 3*, 2062−2063.
40. Wagner, C., and Wagenknecht, H.-A. (2005) Reductive electron transfer in phenothiazine-modified DNA is dependent on the base sequence, *Chem.—Eur. J. 11*, 1871−1876.
41. Amann, N., Huber, R., and Wagenknecht, H.-A. (2004) Phenanthridinium as an artificial base and charge donor in DNA, *Angew. Chem., Int. Ed. 43*, 1845−1847.
42. Huber, R., Amann, N., and Wagenknecht, H.-A. (2004) Synthesis of DNA with phenanthridinium as an artificial DNA base, *J. Org. Chem. 69*, 744−751.
43. Valis, L., Amann, N., and Wagenknecht, H.-A. (2005) Detection of single base mismatches and abasic sites using phenanthridinium as an artificial DNA base and charge donor, *Org. Biomol. Chem. 3*, 36−38.
44. O'Neill, M. A., and Barton, J. K. (2004) DNA-mediated charge transport requires conformational motion of the DNA bases: elimination of charge transport in rigid glasses at 77 K, *J. Am. Chem. Soc. 126*, 13234−13235.
45. O'Neill, M. A., and Barton, J. K. (2004) DNA charge transport: conformationally gated hopping through stacked domains, *J. Am. Chem. Soc. 126*, 11471−11483.
46. Puglisi, J. D., and Tinoco, I., Jr. (1989) Absorbance melting curves of RNA, *Methods Enzymol. 180*, 304−325.
47. Perkampus, H.-H. (1992) *UV−Vis Atlas of Organic Compounds*, 2nd ed., Vol. 2, VCH, Weinheim, Germany.
48. Manoharan, M., Tivel, K. L., Zhao, M., Nafisi, K., and Netzel, T. L. (1995) Base-sequence dependence of emission lifetimes for DNA oligomers and duplexes covalently labeled with pyrene: relative electron-transfer quenching efficiencies of A, G, C, and T nucleosides toward pyrene, *J. Phys. Chem. 99*, 17461−17472.
49. Netzel, T. L., Nafisi, K., Headrick, J., and Eaton, B. E. (1995) Direct observation of photoinduced electron transfer in pyrene-labeled dU nucleosides and evidence for protonated 2′-deoxyuridine anion, dU(H)′, as a primary electron-transfer product, *J. Phys. Chem. 99*, 17948−17955.
50. Netzel, T. L., Zhao, M., Nafisi, K., Headrick, J., Sigman, M. S., and Eaton, B. E. (1995) Photophysics of 2′-deoxyuridine (dU) nucleosides covalently substituted with either 1-pyrenyl or 1-pyrenoyl: observation of pyrene-to-nucleoside charge-transfer emission in 5-(1-pyrenyl)-dU, *J. Am. Chem. Soc. 117*, 9119−9128.
51. Shafirovich, V. Y., Levin, P. P., Kuzmin, V. A., Thorgeirsson, T. E., Kliger, D. S., and Geacintov, N. E. (1994) Photoinduced electron transfer and enhanced triplet yields in benzo[*a*]pyrene derivative-nucleic acid complexes and covalent adducts, *J. Am. Chem. Soc. 116*, 63−72.
52. Shafirovich, V. Y., Courtney, S. H., Ya, N., and Geacintov, N. E. (1995) Proton-coupled photoinduced electron transfer, deuterium isotope effects, and fluorescence quenching in noncovalent benzo-[*a*]pyrenetetraol−nucleoside complexes in aqueous solutions, *J. Am. Chem. Soc. 117*, 4920−4929.
53. Kubota, T., Kano, K., and Konse, T. (1987) Energetics of the sequential electroreduction and electrooxidation steps of benzenoid hydrocarbons, *Bull. Chem. Soc. Jpn. 60*, 3865−3877.
54. Steenken, S., Telo, J. P., Novais, H. M., and Candeias, L. P. (1992) One-electron-reduction potentials of pyrimidine bases, nucleosides, and nucleotides in aqueous solution. Consequences for DNA redox chemistry, *J. Am. Chem. Soc. 114*, 4701−4709.
55. Steenken, S. (1992) Electron-transfer-induced acidity/basicity and reactivity changes of purine and pyrimidine bases. Consequences of redox processes for DNA base pairs, *Free Radical Res. Commun. 16*, 349−379.
56. Chen, T., Cook, G. P., Koppisch, A. T., and Greenberg, M. M. (2000) Investigation of the origin of the sequence selectivity for the 5-halo-2′-deoxyuridine sensitization of DNA to damage by UV-irradiation, *J. Am. Chem. Soc. 122*, 3861−3866.
57. Rivera, E., and Schuler, R. H. (1983) Intermediates in the reduction of 5-halouracils by e_{aq}^{-1}, *J. Phys. Chem. 87*, 3966−3971.
58. Ito, T., and Rokita, S. E. (2003) Excess electron transfer from an internally conjugated aromatic amine to 5-bromo-2′-deoxyuridine in DNA, *J. Am. Chem. Soc. 125*, 11480−11481.
59. Ito, T., and Rokita, S. E. (2004) Criteria for efficient transport of excess electrons in DNA, *Angew. Chem., Int. Ed. 43*, 1839−1842.
60. Ito, T., and Rokita, S. E. (2004) Reductive electron injection into duplex DNA by aromatic amines, *J. Am. Chem. Soc. 126*, 15552−15559.

61. Harriman, A., Millward, G. R., Neta, P., Richoux, M. C., and Thomas, J. M. (1988) Interfacial electron-transfer reactions between platinum colloids and reducing radicals in aqueous solution, *J. Phys. Chem. 92*, 1286−1290.
62. Guilford Jones, I., and Malba, V. (1985) Pyridinium quenchers of $Ru(bpy)_3^{2+}$*. Charge effects of the yield of electron transfer, *J. Org. Chem. 50*, 5776−5782.
63. Behmoras, T., Toulme, J.-J., and Hélène, C. (1981) Specific recognition of apurinic sites in DNA by a tryptophan-containing peptide, *Proc. Natl. Acad. Sci. U.S.A. 78*, 926−930.
64. Behmoras, T., Toulme, J.-J., and Hélène, C. (1981) A tryptophan-containing peptide recognizes and cleaves DNA at apurinic sites, *Nature 292*, 858−859.
65. Hélène, C., and Maurizot, J. C. (1981) Interactions of oligopeptides with nucleic-acids, *CRC Crit. Rev. Biochem. 10*, 213−258.
66. Rawesjari, M. R., Montenay-Garestier, T., and Hélène, C. (1987) Does tryptophan intercalate in DNA? A comparative study of peptide binding to alternating and nonalternating A·T sequences, *Biochemistry 26*, 6825−6831.
67. Wetmore, S. D., Boyd, R. J., and Eriksson, L. A. (2001) A theoretical study of 5-halouracils: electron affinities, ionization potentials and dissociation of the related anions, *Chem. Phys. Lett. 343*, 151−158.
68. Li, X., Sevilla, M. D., and Sanche, L. (2003) DFT investigations of dehalogenation of adenine−halouracil base pairs upon low-energy electron attachment, *J. Am. Chem. Soc. 125*, 8916−8920.
69. Kadysh, V. P., Kaminskii, Y. L., Rumyantseva, L. N., Efimova, V. L., and Strandish, J. P. (1992), *Khim. Geterotsikl. Soedin. 10*, 1404−1408.
70. Wagenknecht, H.-A., and Fiebig, T. (2005) Electron transfer and structural dynamics in DNA, in *Charge Transfer in DNA: From Mechanism to Application* (Wagenknecht, H.-A., Ed.) pp 197−223, Wiley-VCH, Weinheim, Germany.
71. DeFilippis, M. R., Murthy, C. P., Broitman, C. P., Weinraub, D., Faraggi, M., and Klapper, M. H. (1991) Electrochemical properties of tyrosine phenoxy and tryptophan indolyl radicals in peptides and amino acid analogues, *J. Phys. Chem. 95*, 3416−3419.
72. Rajski, S. R., Jackson, B. A., and Barton, J. K. (2000) DNA repair: models for damage and mismatch recognition, *Mutat. Res. 447*, 49−72.

BI0504557